CD109: a multifunctional GPI-anchored protein with key roles in tumor progression and physiological homeostasis

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Running title: CD109 is a multifunctional glycoprotein

Abbreviations

ALK, activin receptor-like kinase; CEC, circulating endothelial cell; CT, computed tomography; EGF, epidermal growth factor; ER, endoplasmic reticulum; GPI, glycosylphosphatidylinositol; GRP78, 78-kDa glucose-regulated protein; H&E, hematoxylin and eosin; IHC, Immunohistochemistry; IL, interleukin; JAK, Janus kinase; qPCR, quantitative polymerase chain reaction; SCC, squamous cell carcinoma; STAT3, signal transducer and activator of transcription-3; TGF-β, transforming growth factor-β; WT, wild-type

Abstract

CD109 is a glycosylphosphatidylinositol-anchored glycoprotein and a member of the α_2-macroglobulin/C3,C4,C5 family of thioester-containing proteins first identified as being expressed on blood cells, including activated T cells and platelets, and a subset of $CD34^+$ bone marrow cells containing megakaryocyte progenitors. Although CD109 carries the biallelic platelet-specific alloantigen Gov, the physiological functions or roles of CD109 in human disease remain largely unknown. It was recently demonstrated that CD109 is expressed in many malignant tumors, including various squamous cell carcinomas and adenocarcinomas, and plays a role as a multifunctional co-receptor. CD109 reportedly associates with transforming growth factor (TGF)-β receptors and negatively regulates TGF-β signaling in keratinocytes. Additionally, CD109 is potentially related to signal transducer and activator of transcription-3 signaling and aberrant cell proliferation. In this review, we describe recent evidence of CD109-specific significance in malignant tumors shown in mouse models and human tissues. Furthermore, we discuss the physiological functions of CD109 *in vitro* and *in vivo*, including results of phenotype analyses of CD109-deficient mice exhibiting epidermal hyperplasia and osteopenia.

Key words: carcinogenesis, CD109, exosome, glycosylphosphatidylinositol-anchored protein, malignant tumors, mouse models, STAT3, TGF-β.

CD109 is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein and a member of the α_2-macroglobulin/C3,C4,C5 family of thioester-containing proteins[1-3] and was first identified as a cell-surface antigen detected by a monoclonal antibody raised against a myeloid cell line (KG1a).[4] CD109 is expressed on phytohemagglutinin-activated T cells, thrombin-activated platelets, endothelial cells, a subset of $CD34^+$ bone marrow cells that contains megakaryocyte progenitors, and mesenchymal stem-cell subsets.[4-6] Additionally, CD109 is expressed in normal tissues, such as myoepithelial cells of the mammary, lacrimal, salivary, and bronchial glands, basal cells of the prostate and bronchial epithelia and epidermis, seminiferous tubules of testis, and osteoblasts and osteoclasts in bone.[7-9] The role of CD109 in blood cells was intensively studied in the 1990s, during which it was revealed to carry the biallelic platelet-specific alloantigen $Gov^{a/b}$ (Fig. 1a) responsible for neonatal alloimmune thrombocytopenia[10,11]; however, its physiological function in blood cells remains unclear. We previously reported that CD109-deficient mice exhibit epidermal hyperplasia in skin and osteopenia in bone,[8,9] suggesting possible CD109-related physiological functions *in vivo*. Although previous studies report CD109 expression in various malignant tumors[12-17] and correlation with prognosis in patients harboring a malignant tumor, such as a glioblastoma or lung adenocarcinoma,[16,17] its roles in human disease remain largely unknown. In this review, we describe our views and perspectives on the significance of CD109 in malignant tumors and provide a description of our recent studies showing the involvement of CD109 in skin and bone-tissue homeostasis in CD109-deficient mice. Furthermore, we briefly describe the possibility of developing new anticancer drugs targeting CD109.

CD109 is a membrane protein expressed in malignant tumors

High levels of CD109 expression have been detected in various tumor-cell lines and tumor tissues, including those associated with squamous cell carcinomas (SCCs) of the lung, esophagus, uterus, and oral cavity, adenocarcinomas of the lung and pancreas, breast cancer, glioblastoma, hepatocellular carcinoma, urothelial carcinoma of the urinary bladder, and several types of sarcomas (Table 1).[12-41] Immunohistochemical (IHC) analyses revealed

elevated CD109 expression on tumor cells (Fig. 1b), whereas its expression in many human tumors has also been confirmed by quantitative PCR (qPCR), DNA microarray, and RNA-seq analyses (Table 1). In particular, CD109 expression on the tumor-cell surface was first analyzed by IHC in SCCs (Fig. 1b),[18-26] followed by identification of surface expression on malignant tumors other than those associated with SCCs, such as glioblastoma (Fig. 1b).[16,30] Moreover, studies show that CD109 subcellular localization varies among cancer types, with localization on the cell surface of tumor cells in most cancers, including skin SCC and glioblastoma,[16,24,30] but diffuse expression observed throughout the cytoplasm of tumor cells in some types of sarcomas.[39] Our IHC data using an anti-CD109 monoclonal antibody confirmed CD109 expression on the seminoma cell surface (Fig. 1b).

The correlation between CD109 expression and tumor grade also varies among cancer types. Although CD109 expression is significantly higher in lower-grade SCCs or urothelial carcinomas of the urinary bladder relative to that in higher-grade SCCs or urothelial carcinomas, respectively,[18,22,24-27] its expression is significantly higher in higher-grade glioma, ductal carcinoma of the breast, non-small cell carcinomas of the lung, hepatocellular carcinoma, epithelioid sarcoma, and myxofibrosarcoma as compared with that in lower-grade tumors, respectively.[12,14-16,34,39] Additionally, CD109 expression in normal tissues is strictly controlled and specifically occurs in myoepithelial cells of the mammary, lacrimal, salivary, and bronchial glands, basal cells of the prostate and bronchial epithelia and epidermis, seminiferous tubules of testis, and osteoblasts and osteoclasts in bone.[7-9] These findings suggest that CD109 expression is controlled in normal tissues and associated with tumor development.

CD109 is secreted in its truncated form or as a component of exosomes

CD109 plays a putative role as a secreted protein, as well as a cell-surface marker. Although CD109 is a GPI-anchored protein, it is also a secreted protein that harbors a signal peptide and furinase-cleavage site (Fig. 1a).[42] The 155-kDa core protein of CD109 is linked to GPI, glycosylated in the endoplasmic reticulum (ER), and transferred to the Golgi apparatus

(Fig. 2a).[42] The glycosylated 190-kDa form of CD109 is further glycosylated and cleaved into 180-kDa and 25-kDa fragments by furinase. In addition to cell-surface expression of the 180-kDa/25-kDa CD109 complex and enrichment on lipid rafts, the 180-kDa CD109 subunit is secreted into culture medium.[42] Additionally, CD109 glycosylation is altered in different cell types, with the manner of secretion also varying between normal or tumor tissue.[43] We recently reported that CD109 is expressed on exosomes,[44] which are extracellular vesicles that range from 50 nm to 100 nm in diameter. Western blot using anti-CD109 antibodies confirmed the presence of 180-kDa and 25-kDa CD109 fragments in an exosome fraction, and immuno-electron microscopy using an anti-CD109 antibody revealed exosome localization of CD109 fragments, suggesting that some 180-kDa/25-kDa complex fragments are released into the extracellular space as exosomal proteins (Fig. 2b).[44] Moreover, CD109 is reportedly highly expressed in a subtype of circulating endothelial cells (CECs),[29] which were recently considered a biomarker of cancer angiogenesis.[45] Furthermore, a previous study reported detection of soluble CD109 in in mouse serum and urine (Table 2).[46] These findings suggest that CD109 might represent a novel biomarker for CD109-expressing malignant tumors.

CD109 is a multifunctional co-receptor that promotes tumor initiation, progression, and metastasis

Although CD109 function has been extensively studied, its detailed biological role remains unclear. The primary function of CD109 reportedly involves downregulating transforming growth factor (TGF)-β signaling through its binding to TGF-β receptor I [activin receptor-like kinase (ALK)5],[47] TGF-β,[48] ALK1,[49] and 78-kDa glucose-regulated protein (GRP78)[50] *in vitro*. Both GPI-anchored CD109 and soluble CD109 bind to TGF-β receptors and attenuate TGF-β-induced SMAD2/3 phosphorylation (Fig. 3a).[42,48,51] Particularly in SCCs, CD109 promotes tumor initiation by suppressing the TGF-β/SMAD/ nuclear factor erythroid 2–related factor-2 pathway[52]; however, CD109 deficiency has no significant effect on TGF-β signaling in mouse keratinocytes *in vitro* and *in vivo*,[8] suggesting that the function

of CD109 in TGF-β signaling is dependent upon cell type, although the reason for this is not yet fully understood.

We previously reported that CD109 enhances epidermal growth factor (EGF) signaling in glioblastoma cells.[43] Furthermore, CD109 also regulates a signal transducer and activator of transcription-3 (STAT3)-related signaling pathway.[8,17,53] A previous study suggested that CD109 interacts with gp130, a component of the interleukin (IL)-6 receptor, to enhance STAT3 phosphorylation and metastasis of A549 lung adenocarcinoma cells, leading to the hypothesis that CD109 is critical for Janus kinase (JAK)-STAT3 signaling in lung adenocarcinoma metastasis.[17] However, further investigations are necessary to confirm a role for CD109 in the JAK-STAT3 pathway. Our previous experiments revealed enhancement of STAT3 phosphorylation in CD109-deficient keratinocytes.[8] Moreover, to evaluate a possible interaction between CD109 and gp130, we performed IL-6-stimulation experiments using CD109-deficient A549 lung adenocarcinoma cells, finding that CD109 deficiency had no apparent effect on STAT3 phosphorylation (Fig. 3b). These controversial results suggest that STAT3 phosphorylation is regulated by CD109 in a more complicated manner than that observed in TGF-β signaling or in a manner different according to cell type. On the other hand, we reported no significant differences in TGF-β, EGF, and STAT3 signaling between CD109-high and -low gliomas, although CD109 is expressed on a population of perivascular brain tumor stem cells and clearly promotes tumor progression.[16] Our data suggested that CD109 exerts functions related to tumor stem cells rather that those associated with regulating TGF-β, EGF, and STAT3 signaling in gliomas.[16]

In addition to its relationship with SCCs, glioblastoma, and lung adenocarcinoma, CD109 has prognostic significance in other human tumors. Previous reports (Table 1) and our analyses using The Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov/) demonstrate that patients with higher expression of CD109 (CD109-high) have a significantly worse prognosis than those with lower expression of CD109 (CD109-low) in various malignant tumors, such as those associated with glioma, glioblastoma, breast ductal carcinoma, lung, gastric, colorectal, and pancreatic adenocarcinomas, as well as urothelial

carcinoma of the urinary bladder, hepatocellular carcinoma, epithelioid sarcoma, myxofibrosarcoma, and diffuse large B cell lymphoma (Table 1 and Fig. 4).[12-17,39,40] A controversial exception is urothelial carcinoma of the urinary bladder, where CD109 expression inversely correlated with poor prognosis in patients in our study (Table 1).[27] Conversely, there was no significant difference in prognosis between CD109-high and -low patients with SCCs (Table 1 and Fig. 4a).[18] These results suggest that the role of CD109 in tumor progression or metastasis in SCCs is distinct from that in other tumor types.

The role of CD109 *in vivo*

As noted, studies using cultured tumor cells and clinicopathologic analyses revealed a role for CD109 in modulating TGF-β signaling by binding to its cognate receptors, and that CD109 expression correlates with the outcomes of various human malignant tumors. Additionally, *in vivo* CD109 functions have also been studied using several mouse models (Table 2).[8,9,16,17,46,52,54-56] Among these studies, we compared the phenotype of CD109-deficient mice with that of their wild-type (WT) counterparts,[8,9] with macroscopic analyses revealing transient impairment of hair growth in CD109-deficient mice (Fig. 5). Subsequent histologic analysis with hematoxylin and eosin (H&E) staining revealed persistent hyperplasia of the epidermis and sebaceous glands, and IHC analysis revealed thickening of the basal and suprabasal layers of the epidermis (along with p63-positivity) in CD109-deficient mice (Fig. 5).[8] Additionally, CD109-deficient mice exhibited an osteopenia phenotype that was verified by reduced bone/tissue volume, decreased trabecular number, and increased trabecular separation according to micro-computed tomography (CT) scans (Fig. 6a).[9] IHC analysis showed that CD109 is expressed in both osteoblasts and osteoclasts, and serum analyses revealed that CD109 deficiency increases their activities in mice.[9] These findings suggest that CD109-deficient mice exhibit a "high-turnover osteoporosis" phenotype (Fig. 6b).[9] Paradoxically, CD109 deficiency reduced osteoclast formation in pre-osteoclast cells *in vitro*.[9,57] These phenotypes indicated that CD109 plays a regulatory role in skin and bone homeostasis. In the skin, previous studies analyzed dermal fibrosis induced by bleomycin and

in vivo wound healing using K14-CD109 transgenic mice.[54-56] Their results suggested that K14-CD109 transgenic mice were resistant to bleomycin-induced skin fibrosis and showed reduced dermal thickness and decreased fibrotic response in wounds as compared with WT mice. Interestingly, these results were accompanied by increases in STAT3 signaling in CD109-deficient mice[8] and decreases in TGF-β signaling in K14-CD109 transgenic mice.[49]

The roles of CD109 in tumors have also been examined *in vivo*. A previous study demonstrated that CD109 promotes the initiation of skin squamous cell tumors exhibiting suppressed TGF-β signaling,[52] the progression of gliomas with resistance to chemotherapy,[16] and metastasis of lung adenocarcinoma cells exhibiting enhanced STAT3 phosphorylation[17] in specific mouse models, respectively. These results and the prognostic significance of CD109 in human tumors suggest CD109 as not only a prognostic marker but also as having a potentially important biological role related to poor prognosis in patients with malignant tumors.

Further questions and perspectives

This review describes the significant roles of CD109 in malignant tumors, as well as the molecular mechanisms by which CD109 promotes tumor initiation, progression, and metastasis and/or regulates physiological homeostasis in skin and bone. However, there remain a number of unanswered biological questions. For example, the roles of CD109 in TGF-β and STAT3 signaling are controversial, although numerous studies report their involvement in CD109-mediated promotion of malignancy in various tumors or the suppression of skin fibrosis *in vivo*. In particular, the effect of CD109 on STAT3 phosphorylation remains to be elucidated. Furthermore, both the CD109-binding partner and the direction of regulation (up or down) of STAT3 signaling by CD109 remain unclear.

Another provocative question concerns whether CD109 functions are limited to regulating TGF-β, EGF, and STAT3 signaling. A preliminary study reported that CD109 might regulate tumor necrosis factor-α signaling in keratinocytes[58]; however, the biological role of CD109 as a multifunctional co-receptor requires further clarification.

Although numerous studies have described CD109 functions or the underlying molecular mechanisms, its precise role in blood cells remains uncertain. As described, CD109 was first identified in a myeloid cell line (KG1a) and subsequently demonstrated as playing a critical role in hematological disorders, such as neonatal alloimmune thrombocytopenia, involving the platelet-specific alloantigen Gov$^{a/b}$. Unfortunately, no relevant findings were reported involving blood cells from CD109-deficient mice.[8,9] Although reports indicated that CD109 regulates osteoclastogenesis and is a putative risk marker associated with diffuse large B cell lymphoma,[40,57] the physiological functions of CD109 in blood cells, as well as in skin and bone, require additional investigation.

CD109 is potentially advantageous for drug development not only based on its expression on the cell surface and ability to bind the anti-CD109 antibody *in vivo* but also because CD109-deficient mice exhibit no lethal phenotype, with no significant difference in life span between WT and CD109-deficient mice.[8] Although there are no reports that the anti-CD109 antibody acts as a therapeutic agent *in vivo*, one study showed that CD109 expression was sufficient to induce antibody internalization and cell killing in 11q13-amplified cell lines via an antibody/drug-conjugate approach.[59] Further research is needed to develop tumor treatments involving antibody based drugs targeting CD109.

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Disclosure statement

None declared.

Author contributions

SM and AE contributed to the conception and design of the study. SM, YS, and TT acquired and analyzed the data. SM, AE, and YS drafted the manuscript and figures. SM, AE, YM, and MT reviewed the manuscript. MT supervised the study.

References

1 Lin M, Sutherland DR, Horsfall W, et al. Cell surface antigen CD109 is a novel member of the α_2 macroglobulin/C3, C4, C5 family of thioester-containing proteins. *Blood* 2002; **99**: 1683-91.

2 Solomon KR, Sharma P, Chan M, et al. CD109 represents a novel branch of the α_2-macroglobulin/complement gene family. *Gene* 2004; **327**: 171-83.

3 Janssen BJ, Huizinga EG, Raaijmakers HC, et al. Structures of complement component C3 provide insights into the function and evolution of immunity. *Nature* 2005; **437**: 505-11.

4 Sutherland DR, Yeo E, Ryan A, et al. Identification of a cell-surface antigen associated with activated T lymphoblasts and activated platelets. *Blood* 1991; **77**: 84-93.

5 Murray LJ, Bruno E, Uchida N, et al. CD109 is expressed on a subpopulation of CD34+ cells enriched in hematopoietic stem and progenitor cells. *Exp Hematol* 1999; **27**: 1282-94.

6 Giesert C, Marxer A, Sutherland DR, et al. Antibody W7C5 defines a CD109 epitope expressed on CD34+ and CD34- hematopoietic and mesenchymal stem cell subsets. *Ann N Y Acad Sci* 2003; **996**: 227-30.

7 Hasegawa M, Hagiwara S, Sato T, et al. CD109, a new marker for myoepithelial cells of mammary, salivary, and lacrimal glands and prostate basal cells. *Pathol Int* 2007; **57**: 245-50.

8 Mii S, Murakumo Y, Asai N, et al. Epidermal hyperplasia and appendage abnormalities in mice lacking CD109. *Am J Pathol* 2012; **181**: 1180-9.

9 Mii S, Hoshino A, Enomoto A, et al. CD109 deficiency induces osteopenia with an osteoporosis-like phenotype in vivo. *Genes Cells* 2018; **23**:590-8.

10 Smith JW, Hayward CP, Horsewood P, et al. Characterization and localization of the $Gov^{a/b}$ alloantigens to the glycosylphosphatidylinositol-anchored protein CDw109 on human platelets. *Blood* 1995; **86**: 2807-14.

11 Schuh AC, Watkins NA, Nguyen Q, et al. A tyrosine703serine polymorphism of CD109 defines the Gov platelet alloantigens. *Blood* 2002; **99**: 1692-8.

12 Tao J, Li H, Li Q, et al. CD109 is a potential target for triple-negative breast cancer. *Tumour Biol* 2014; **35**: 12083-90.

13 Yi JM, Dhir M, Van Neste L, et al. Genomic and epigenomic integration identifies a prognostic signature in colon cancer. *Clin Cancer Res* 2011; **17**: 1535-45.

14 Zong G, Xu Z, Zhang S, et al. CD109 mediates cell survival in hepatocellular carcinoma cells. *Dig Dis Sci* 2016; **61**: 2303-14.

15 Emori M, Tsukahara T, Murase M, et al. High expression of CD109 antigen regulates the phenotype of cancer stem-like cells/cancer-initiating cells in the novel epithelioid sarcoma cell line ESX and is related to poor prognosis of soft tissue sarcoma. *PLoS One* 2013; **8**: e84187.

16 Shiraki Y, Mii S, Enomoto A, et al. Significance of perivascular tumour cells defined by CD109 expression in progression of glioma. *J Pathol* 2017; **243**: 468-80.

17 Chuang CH, Greenside PG, Rogers ZN, et al. Molecular definition of a metastatic lung cancer state reveals a targetable CD109-Janus kinase-Stat axis. *Nat Med* 2017; **23**: 291-300.

18 Hagiwara S, Murakumo Y, Sato T, et al. Up-regulation of CD109 expression is associated with carcinogenesis of the squamous epithelium of the oral cavity. *Cancer Sci* 2008; **99**: 1916-23.

19 Ni RS, Shen X, Qian X, et al. Detection of differentially expressed genes and association with clinicopathological features in laryngeal squamous cell carcinoma. *Oncol Lett* 2012; **4**: 1354-60.

20 Jia W, Ren C, Wang L, et al. CD109 is identified as a potential nasopharyngeal carcinoma biomarker using aptamer selected by cell-SELEX. *Oncotarget* 2016; **7**: 55328-42.

21 Hashimoto M, Ichihara M, Watanabe T, et al. Expression of CD109 in human cancer. *Oncogene* 2004; **23**: 3716-20.

22 Dong F, Liu F, Yan S, et al. Elevated Expression of CD109 in esophageal squamous cell carcinoma. *Pathol Oncol Res* 2015; **21**: 1273-5.

23 Zhang JM, Hashimoto M, Kawai K, et al. CD109 expression in squamous cell carcinoma of the uterine cervix. *Pathol Int* 2005; **55**: 165-9.

24 Özbay PÖ, Ekinci T, Yiğit S, et al. Investigation of prognostic significance of CD109 expression in women with vulvar squamous cell carcinoma. *Onco Targets Ther* 2013; **6**: 621-7.

25 Dong F, Wang Y, Li L, et al. CD109 expression is increased in cutaneous squamous cell carcinoma. *J Dermatol* 2014; **41**: 947-9.

26 Dong F, Wang J, Xu Y, et al. CD109 expression is upregulated in penile squamous cell carcinoma. *Oncol Lett* 2017; **14**: 6012-6.

27 Hagikura M, Murakumo Y, Hasegawa M, et al. Correlation of pathological grade and tumor stage of urothelial carcinomas with CD109 expression. *Pathol Int* 2010; **60**: 735-43.

28 Ohshima Y, Yajima I, Kumasaka MY, et al. CD109 expression levels in malignant melanoma. *J Dermatol* Sci 2010; **57**: 140-2.

29 Cuppini L, Calleri A, Bruzzone MG, et al. Prognostic value of CD109+ circulating endothelial cells in recurrent glioblastomas treated with bevacizumab and irinotecan. *PLoS One* 2013; **8**: e74345.

30 Minata M, Audia A, Shi J, et al. Phenotypic Plasticity of Invasive Edge Glioma Stem-like Cells in Response to Ionizing Radiation. *Cell Rep* 2019; 26: 1893-905.

31 Hasegawa M, Moritani S, Murakumo Y, et al. CD109 expression in basal-like breast carcinoma. *Pathol Int* 2008; **58**: 288-94.

32 Sato T, Murakumo Y, Hagiwara S, et al. High-level expression of CD109 is frequently detected in lung squamous cell carcinomas. *Pathol Int* 2007; **57**: 719-24.

33 Shukla S, Evans JR, Malik R, et al. Development of a RNA-seq based prognostic signature in lung adenocarcinoma. *J Natl Cancer Inst* 2017; **109**.

34 Zhang F, Lin H, Gu A, et al. SWATH^{TM}- and iTRAQ-based quantitative proteomic analyses reveal an overexpression and biological relevance of CD109 in advanced NSCLC. *J Proteomics* 2014; **102**: 125-36.

35 Haun RS, Fan CY, Mackintosh SG, et al. CD109 overexpression in pancreatic cancer identified by cell-surface glycoprotein capture. *J Proteomics Bioinform* 2014; **Suppl 10**: S10003.

36 Moffitt RA, Marayati R, Flate EL, et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. *Nat Genet* 2015; **47**: 1168-78.

37 Arias-Pinilla GA, Dalgleish AG, Mudan S, et al. Development of novel monoclonal antibodies against CD109 overexpressed in human pancreatic cancer. *Oncotarget* 2018; **9**: 19994-20007.

38 Dong F, Lu C, Chen X, et al. CD109 is a novel marker for squamous cell/adenosquamous carcinomas of the gallbladder. *Diagn Pathol* 2015; **10**: 137.

39 Emori M, Tsukahara T, Murata K, et al. Prognostic impact of CD109 expression in myxofibrosarcoma. *J Surg Oncol* 2015; **111**: 975-9.

40 Yokoyama M, Ichinoe M, Okina S, et al. CD109, a negative regulator of TGF-β signaling, is a putative risk marker in diffuse large B-cell lymphoma. *Int J Hematol* 2017; **105**: 614-22.

41 Dong F, Cheng Y, Sun Q, et al. CD109 is specifically expressed in endothelial cells of cutaneous cavernous haemangioma. *Histopathology* 2015; **67**: 133-5.

42 Hagiwara S, Murakumo Y, Mii S, et al. Processing of CD109 by furin and its role in the regulation of TGF-β signaling. *Oncogene* 2010; **29**: 2181-91.

43 Zhang JM, Murakumo Y, Hagiwara S, et al. CD109 attenuates TGF-β1 signaling and enhances EGF signaling in SK-MG-1 human glioblastoma cells. *Biochem Biophys Res Commun* 2015; **459**: 252-8.

44 Sakakura H, Mii S, Hagiwara S, et al. CD109 is a component of exosome secreted from cultured cells. *Biochem Biophys Res Commun* 2016; **469**: 816-22.

45 Bertolini F, Shaked Y, Mancuso P, et al. The multifaceted circulating endothelial cell in cancer: towards marker and target identification. *Nat Rev Cancer* 2006; **6**: 835-45.

46 Sakakura H, Murakumo Y, Mii S, et al. Detection of a soluble form of CD109 in serum of CD109 transgenic and tumor xenografted mice. *PLoS One* 2014; **9**: e83385.

47 Bizet AA, Liu K, Tran-Khanh N, et al. The TGF-β co-receptor, CD109, promotes internalization and degradation of TGF-β receptors. *Biochim Biophys Acta* 2011; **1813**: 742-53.

48 Li C, Hancock MA, Sehgal P, et al. Soluble CD109 binds TGF-β and antagonizes TGF-β signalling and responses. *Biochem J* 2016; **473**: 537-47.

49 Vorstenbosch J, Nguyen CM, Zhou S, et al. Overexpression of CD109 in the Epidermis differentially regulates ALK1 versus ALK5 signaling and modulates extracellular matrix synthesis in the skin. *J Invest Dermatol* 2017; **137**: 641-9.

50 Tsai YL, Ha DP, Zhao H, et al. Endoplasmic reticulum stress activates SRC, relocating chaperones to the cell surface where GRP78/CD109 blocks TGF-β signaling. *Proc Natl Acad Sci USA* 2018; **115**: E4245-54.

51 Finnson KW, Tam BY, Liu K, et al. Identification of CD109 as part of the TGF-β receptor system in human keratinocytes. *FASEB J* 2006; **20**: 1525-7.

52 Sunagawa M, Mii S, Enomoto A, et al. Suppression of skin tumorigenesis in CD109-deficient mice. *Oncotarget* 2016; **7**: 82836-50.

53 Litvinov IV, Bizet AA, Binamer Y, et al. CD109 release from the cell surface in human keratinocytes regulates TGF-β receptor expression, TGF-β signalling and STAT3 activation: relevance to psoriasis. *Exp Dermatol* 2011; **20**: 627-32.

54 Vorstenbosch J, Al-Ajmi H, Winocour S, et al. CD109 overexpression ameliorates skin fibrosis in a mouse model of bleomycin-induced scleroderma. *Arthritis Rheum* 2013; **65**: 1378-83.

55 Vorstenbosch J, Gallant-Behm C, Trzeciak A, et al. Transgenic mice overexpressing CD109 in the epidermis display decreased inflammation and granulation tissue and

improved collagen architecture during wound healing. *Wound Repair Regen* 2013; **21**: 235-46.

56 Winocour S, Vorstenbosch J, Trzeciak A, et al. CD109, a novel TGF-β antagonist, decreases fibrotic responses in a hypoxic wound model. *Exp Dermatol* 2014; **23**: 475-9.

57 Wang Y, Inger M, Jiang H, et al. CD109 plays a role in osteoclastogenesis. *PLoS One* 2013; **8**: e61213.

58 Nguyen C, Finnson K, Philip A. CD109 regulates TNF-α signaling in human keratinocytes. *Can J Plast Surg* 2014; **22**: 123.

59 Hoover H, Li J, Marchese J, et al. Quantitative proteomic verification of membrane proteins as potential therapeutic targets located in the 11q13 amplicon in cancers. *J Proteome Res* 2015; **14**: 3670-9.

60 Pierleoni A, Martelli PL, Casadio R. PredGPI: a GPI-anchor predictor. *BMC Bioinformatics* 2008; **9**: 392.

61 Eisenhaber B, Bork P, Eisenhaber F. Prediction of potential GPI-modification sites in proprotein sequences. *J Mol Biol* 1999; **292**: 741-58.

Table 1 CD109 expression and its prognostic significance in human tumors.

	Methods for human tissue analysis*	Correlation with histological grade†	Involvement in tumor progression	Correlation with prognosis‡
Head and neck SCCs				
SCC of the oral cavity[18]	IHC(m/c)	Higher expression in lower grade	Increased cell proliferation	N.S.
Laryngeal SCC[19]	DNA microarray	N.S.	—	—
Nasopharyngeal SCC[20]	IHC(m/c)	—	—	—
Esophageal SCC[21,22]	qPCR, IHC(m/c)	Higher expression in lower grade	—	—
Cervical SCC[23]	qPCR	N.S.	—	—
Skin cancer				
SCC[24-26]	IHC(m/c)	Higher expression in lower grade	—	—
Malignant melanoma[28]	IHC(m/c)	—	—	—
Brain tumor				
Glioma[16]	IHC(m/c), ISH	Higher expression in higher grade	Highly expression in $CD44^+$ glioma stem-like cells	Worse in CD109-high group
Glioblastoma[16,29,30]	FCM(CECs), IHC(m/c)	—		
Breast cancer				
Ductal carcinoma[12]	IHC(m/c)	Higher expression in higher grade	Increased proliferation of $CD44^+$ cancer stem-like cells	Worse in CD109-high group
Basal-like carcinoma[12,31]	IHC(m/c)	N.S.		—
Lung cancer				
SCC[21,32]	qPCR, IHC(m/c)	—	—	—
Adenocarcinoma[21,32,33]	qPCR, IHC(c/m), RNA-seq	—	—	—
Non-small cell carcinomas[34]	ELISA(serum), qPCR	Higher expression in higher grade	Increased cell proliferation	—
Gastric adenocarcinoma[21]	qPCR	—	—	—
Colorectal adenocarcinoma[13]	Methylation-specific PCR	N.S.	—	Worse in CD109-methylated group
Pancreatic adenocarcinoma[35-37]	IHC(c/m), DNA microarray	N.S.	—	—
Hepatocellular carcinoma[14]	IHC(c)	Higher expression in higher grade	Increased cell proliferation	Worse in CD109-high group
Squamous cell / adenosquamous carcinomas of the gallbladder[38]	IHC(m/c)	—	—	—
Urothelial carcinoma of the urinary bladder[27]	IHC(m/c)	Higher expression in lower grade	Similar expression pattern to CD44	Worse in CD109-low group
Sarcoma				
Epithelioid sarcoma[15]	IHC(c/m)	Higher expression in higher grade	Highly expression in	Worse in CD109-high group

Myxofibrosarcoma[39]	IHC(c/m)	Higher expression in higher grade	ALDH1^{high} tumor stem-like cells —	Worse in CD109-high group
Diffuse large B-cell lymphoma[40]	IHC(c/m)	—	—	Worse in CD109-high group
Seminoma (Fig. 1b)	IHC(m)	—	—	—
Hemangioma[41]	IF, IHC(m/c)	—	—	—

[*] m, membrane staining; m/c, membrane and cytoplasmic staining (membrane staining is stronger than cytoplasmic staining); c/m, cytoplasmic and membrane staining (cytoplasmic staining is stronger than membrane staining); c, cytoplasmic staining.

[†]Histopathological grade was assigned according to the TNM Classification of Malignant Tumours, except for brain tumors, where the World Health Organization grading system was used.

[‡]This column excludes analyses using TCGA data in each article.

ALDH1, aldehyde dehydrogenase isoform 1; ELISA, enzyme-linked immunosorbent assay; FCM, flow cytometry; IF, immunofluorescence; ISH, *in situ* hybridization; N.S. not significant.

Table 2 Studies of CD109 using mouse models.

	Analyzed mouse models	Results	CD109 involvement in disease progression
Skin			
Epidermis[8]	CD109-deficient mice	Psoriasis-like phenotype with epidermal hyperplasia in CD109-deficient mice	—
Fibrosis[54]	CD109-transgenic mice with bleomycin-induced fibrosis	Resistance to bleomycin-induced skin fibrosis in CD109-transgenic mice	Suppression of TGF-β signaling
Wound healing[55,56]	CD109-transgenic mice	Reduced dermal thickness in excisional wounds in CD109-transgenic mice Decreased fibrotic response in hypoxic wounds in CD109-transgenic mice	Suppression of TGF-β signaling
Carcinogenesis[52]	CD109-deficient mice with DMBA/TPA-induced carcinogenesis	Reduced skin tumorigenesis by CD109 deficiency	Suppression of TGF-β signaling
Bone[9]	CD109-deficient mice	Osteoporosis-like phenotype with high bone turnover in CD109-deficient mice	—
Brain tumorigenesis[16]	CD109-deficient mice (RCAS/tv-a system)	Lower survival rate in WT mice as compared with CD109-deficient mice	Increased chemoresistance
Lung adenocarcinoma[17]	KP mice	Reduced lung metastases of CD109-knockdown cells	Enhancement of JAK/STAT3 pathway
Biomarker for tumors[46]	Xenografted mouse model	CD109 detected in the serum and urine of model mice.	—

DMBA, 7,12-dimethylbenz (α) anthracene; KP mice, *Kras*$^{LSL\text{-}G12D/WT}$*; p53*$^{flox/flox}$ mice; RCAS, replication-competent avian leukosis virus splice acceptor; TPA, tetradecanoyl-phorbol acetate; tv-a, subgroup A avian leukosis virus receptor.

Figure legends

Figure 1 Structure of CD109 and its expression in malignant tumors.

(**a**) Schematic illustration of human CD109 protein. The Gov[a] allelic form has tyrosine at amino acid (aa) position 703, whereas the Gov[b] allelic form has serine at the same position. The GPI-anchor attachment site is predicted at aa 1418,[60] 1420,[1] or 1421.[61] (**b**) Representative histological images of skin SCC, lung SCC, glioblastoma, and seminoma. H&E staining (left) and IHC staining with the anti-CD109 antibody (right) in these tumor tissues.

Figure 2 Maturation and secretion of CD109 *in vitro*.

(**a**) The 155-kDa core protein of CD109 is linked to GPI and glycosylated in the ER. CD109 is cleaved into 180-kDa and 25-kDa fragments by furinase in the Golgi apparatus. The complex formed by the 180-kDa and 25-kDa fragments is present on the cell surface. 180-kDa fragments are also secreted into the culture medium. This figure is adapted from Hagiwara, et al. (2010)[42] and presented in accordance with Springer *Nature* author reuse guidelines. (**b**) Some 180-kDa/25-kDa complexes are released into the extracellular space as exosomal proteins. This figure is adapted from Sakakura et al.[44] (CD109 is a component of exosome secreted from cultured cells, *Biochem Biophys Res Commun* 2016;469:816–822) with permission from Elsevier.

Figure 3 The role of CD109 in cellular signaling.

(**a**) CD109 negatively regulates TGF-β signaling in keratinocytes. sCD109, soluble CD109; TGFβRI, TGF-β receptor I; TGFβRII, TGF-β receptor II. (**b**) CD109 plays paradoxical roles in STAT3 signaling. No apparent differences were observed in STAT3 phosphorylation between CD109-knockout (KO) A549 cells and control cells, whereas previous reports suggest that CD109 regulates STAT3 phosphorylation. CD109 KO in A549 cells was performed using the CRISPR/Cas9 method, as previously described,[17] but with the following guide RNA sequences: CCCGGAGGAAATGTGACTAT (A) and ATTTATGAGCTACGTGTAAC (B).

Figure 4 CD109 expression correlates with the prognosis of patients with glioblastomas, lung adenocarcinomas, and gastric adenocarcinomas.
A total of 520 head and neck SCC cases (**a**), 164 glioblastoma cases (**b**), 504 lung adenocarcinoma cases (**c**), and 387 gastric adenocarcinoma cases (**d**) from the TCGA were classified according to CD109 expression, and overall survival rates were plotted using based on Kaplan–Meier analysis. A cut-off z-score value of 0.3 was selected from the training analysis and used to classify tumors as CD109-high or -low. All analyses in this figure were performed, as previously described.[16]

Figure 5 Skin phenotype of CD109-deficient (*CD109*$^{-/-}$) mice.
CD109$^{-/-}$ mice develop skin abnormalities. Macroscopic images show hair-growth impairment in a *CD109*$^{-/-}$ mouse with a C57BL/6J genetic background at postnatal day ~28. H&E-stained skin sections showing hyperplasia of the epidermis and sebaceous glands. IHC staining for p63 revealed thickening of the basal and suprabasal layers (positive for the basal-cell marker p63).

Figure 6 Bone phenotype of *CD109*$^{-/-}$ mice. (**a**) Quantitative morphological parameters of femurs from WT (*CD109*$^{+/+}$) and *CD109*$^{-/-}$ mice with a 129S6 genetic background and measured by micro-CT scans at 8 weeks of age: bone volume/tissue volume (BV/TV), trabecular number (Tb.N), and trabecular separation (Tb.Sp). These results obtained in 129S6 mice are consistent with those in C57BL/6J mice.[9] Error bars indicate the standard deviation. *$P < 0.05$. (**b**) Schematic illustration of the role of CD109 in bone homeostasis.

Figure 1

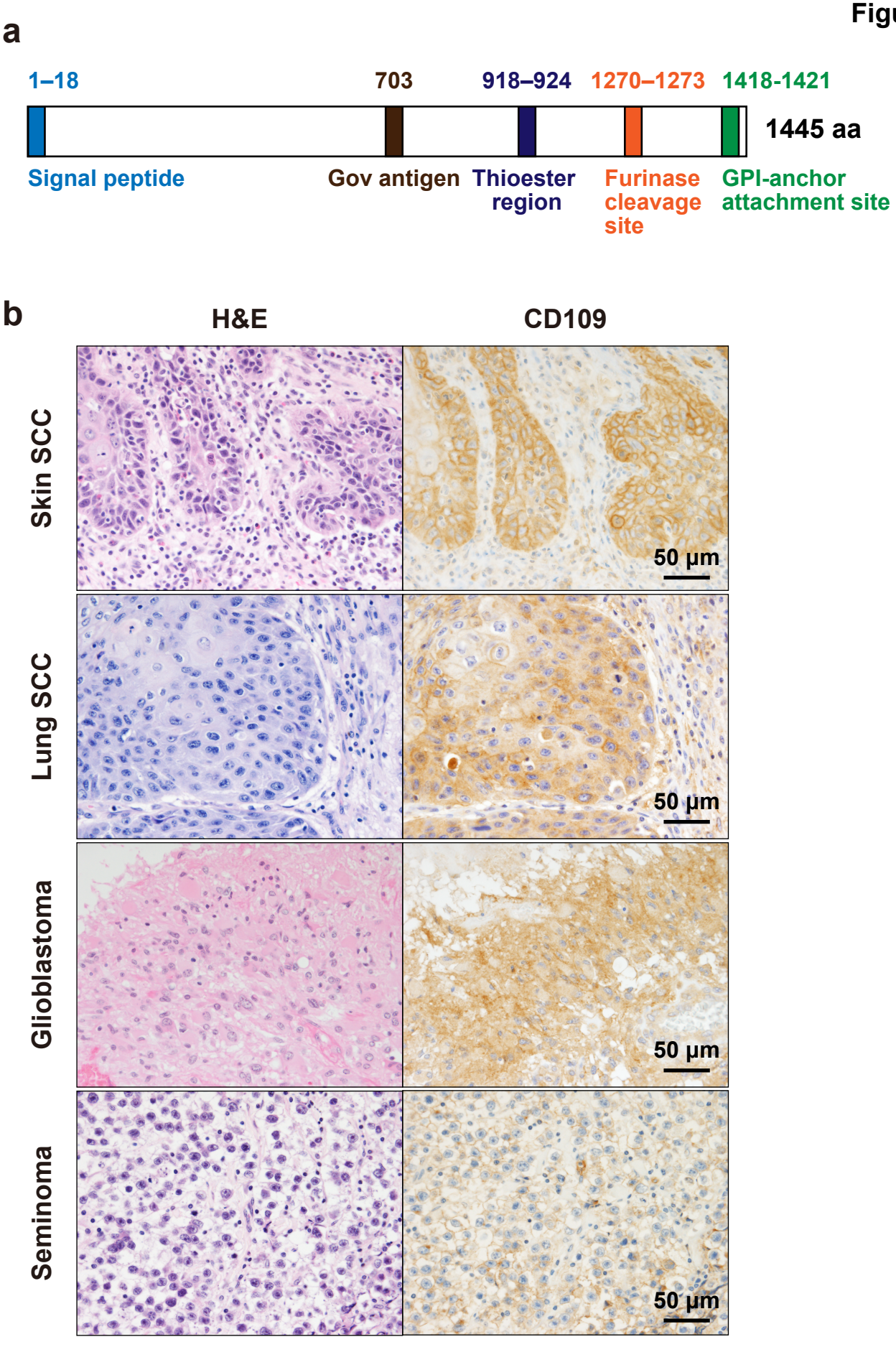

a
1–18
703
918–924
1270–1273
1418-1421
1445 aa
Signal peptide
Gov antigen
Thioester region
Furinase cleavage site
GPI-anchor attachment site
b
H&E
CD109
Skin SCC
Lung SCC
Glioblastoma
Seminoma
50 µm
50 µm
50 µm
50 µm

Figure 2

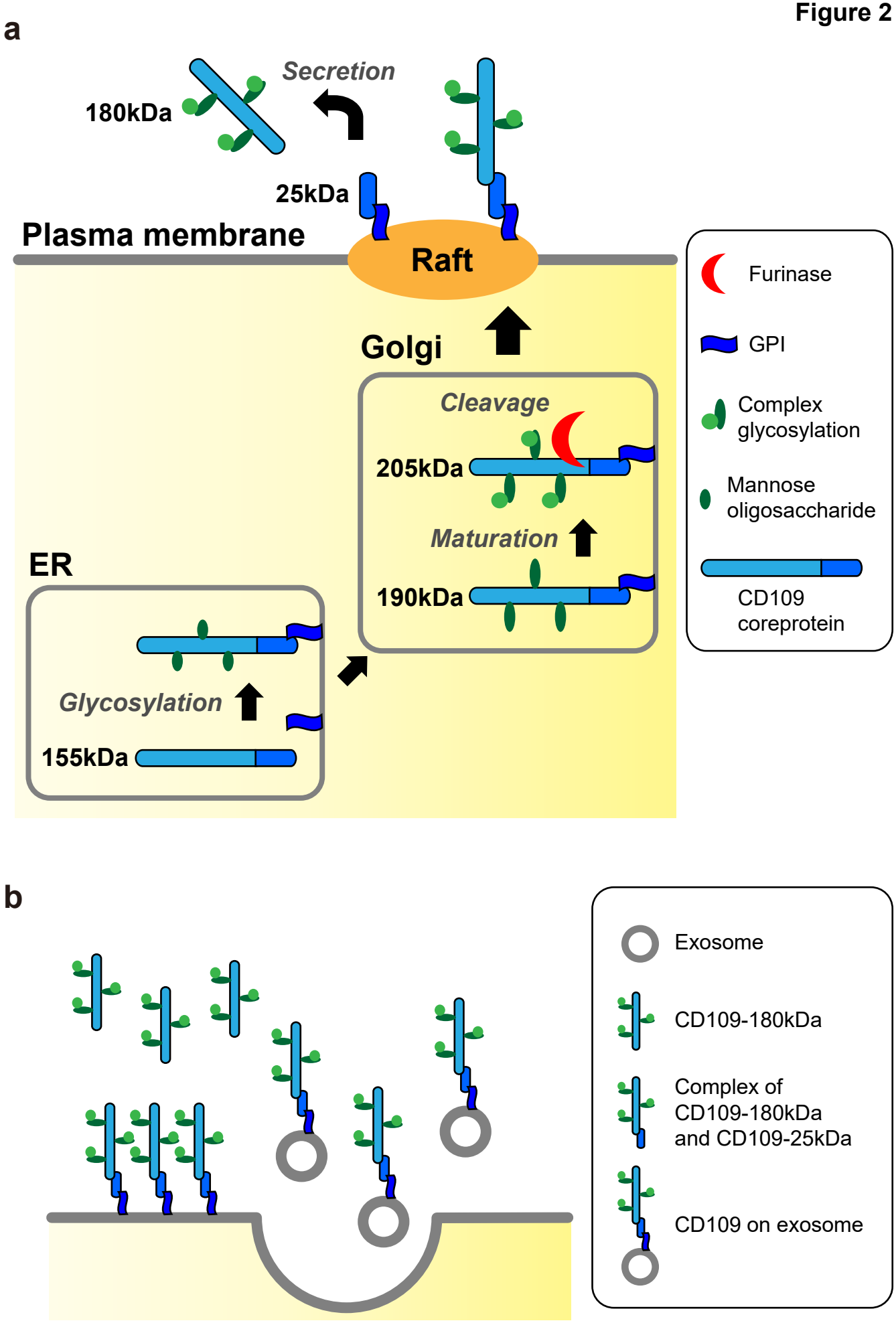
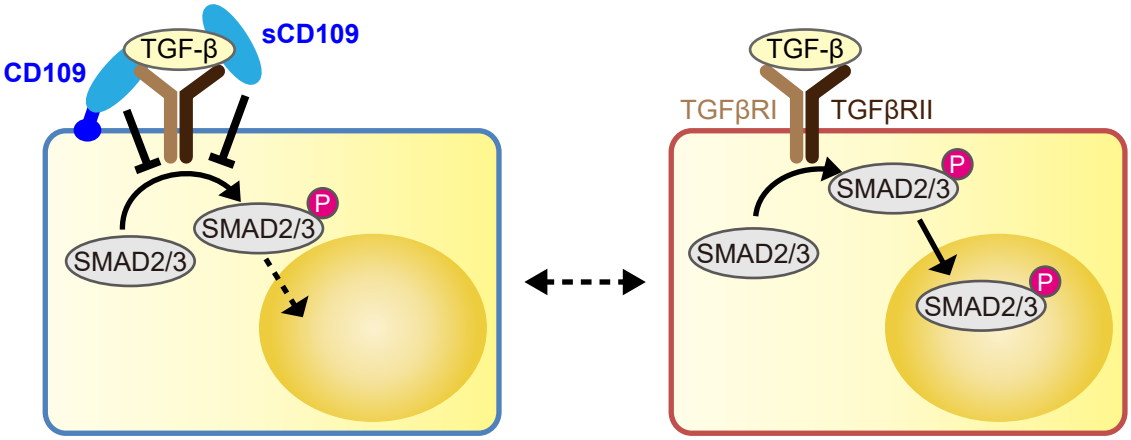

a
Secretion
180kDa
25kDa
Plasma membrane
Raft
Golgi
Cleavage
205kDa
Maturation
190kDa
ER
Glycosylation
155kDa
Furinase
GPI
Complex glycosylation
Mannose oligosaccharide
CD109 coreprotein
b
Exosome
CD109-180kDa
Complex of CD109-180kDa and CD109-25kDa
CD109 on exosome

Figure 3

a


CD109
sCD109
TGF-β
SMAD2/3
P
SMAD2/3
TGF-β
TGFβRI
TGFβRII
SMAD2/3
P
SMAD2/3
P
SMAD2/3

b

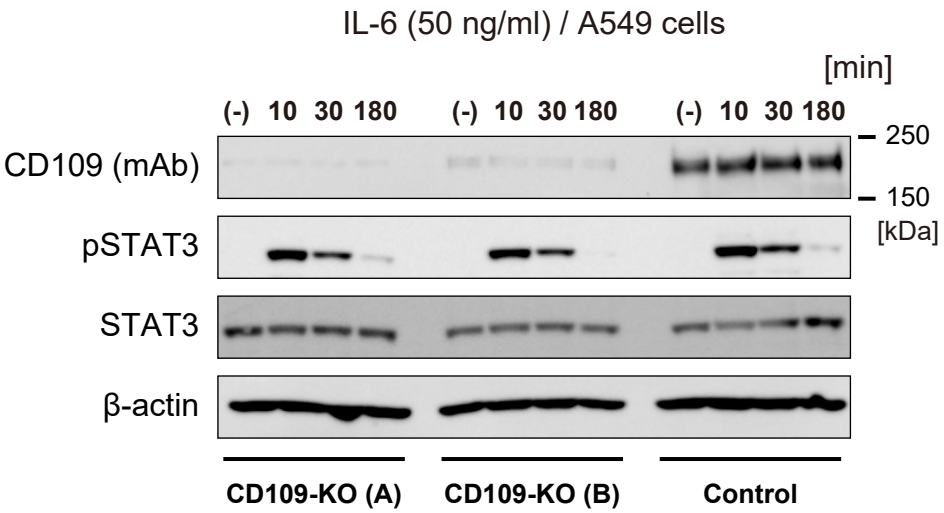

IL-6 (50 ng/ml) / A549 cells
[min]
(-) 10 30 180 (-) 10 30 180 (-) 10 30 180
CD109 (mAb)
pSTAT3
STAT3
β-actin
250
150
[kDa]
CD109-KO (A)
CD109-KO (B)
Control

Figure 4

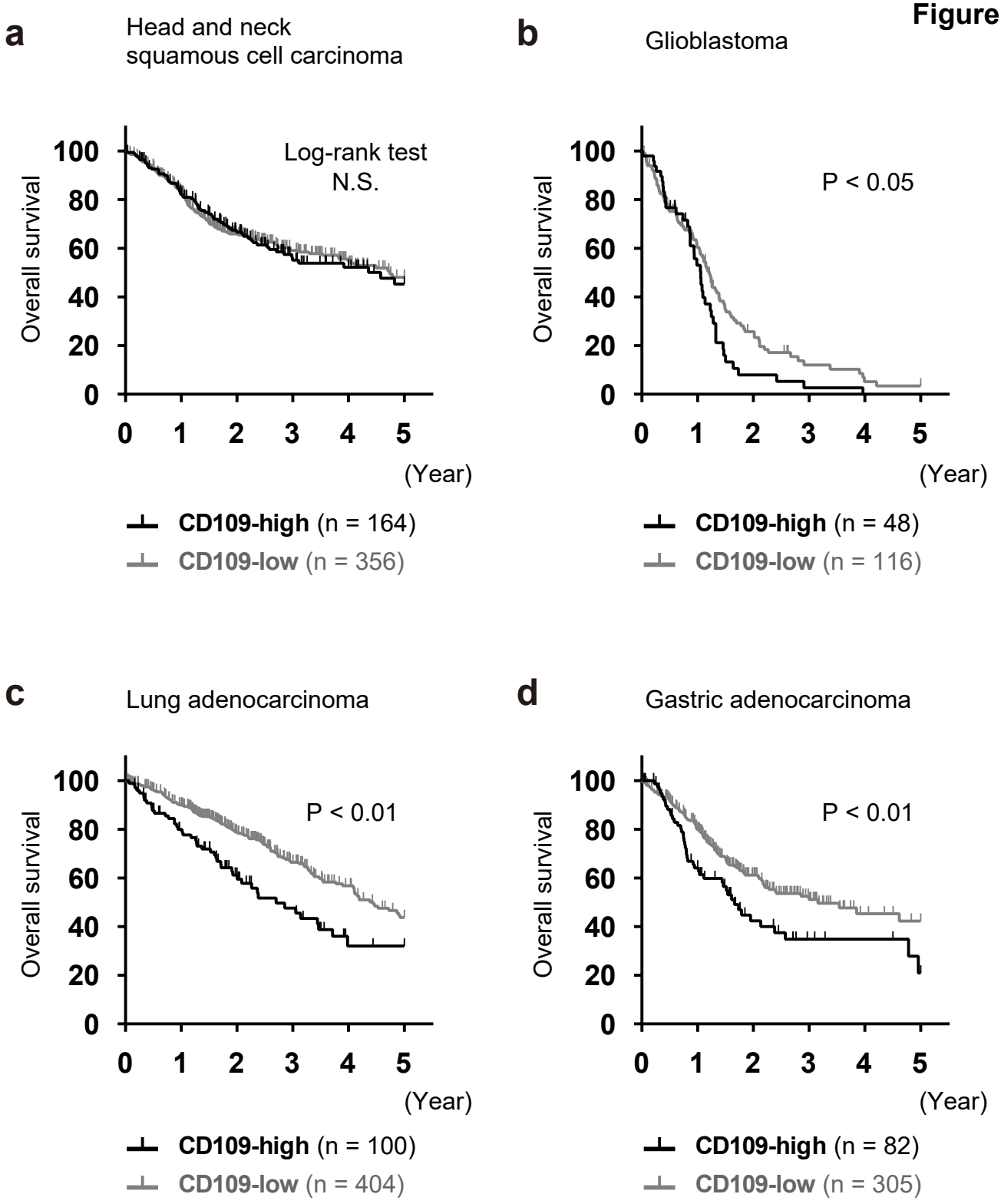

a
Head and neck squamous cell carcinoma
Log-rank test N.S.
Overall survival
100
80
60
40
20
0
0 1 2 3 4 5
(Year)
CD109-high (n = 164)
CD109-low (n = 356)
b
Glioblastoma
P < 0.05
Overall survival
100
80
60
40
20
0
0 1 2 3 4 5
(Year)
CD109-high (n = 48)
CD109-low (n = 116)
c
Lung adenocarcinoma
P < 0.01
Overall survival
100
80
60
40
20
0
0 1 2 3 4 5
(Year)
CD109-high (n = 100)
CD109-low (n = 404)
d
Gastric adenocarcinoma
P < 0.01
Overall survival
100
80
60
40
20
0
0 1 2 3 4 5
(Year)
CD109-high (n = 82)
CD109-low (n = 305)

Figure 5

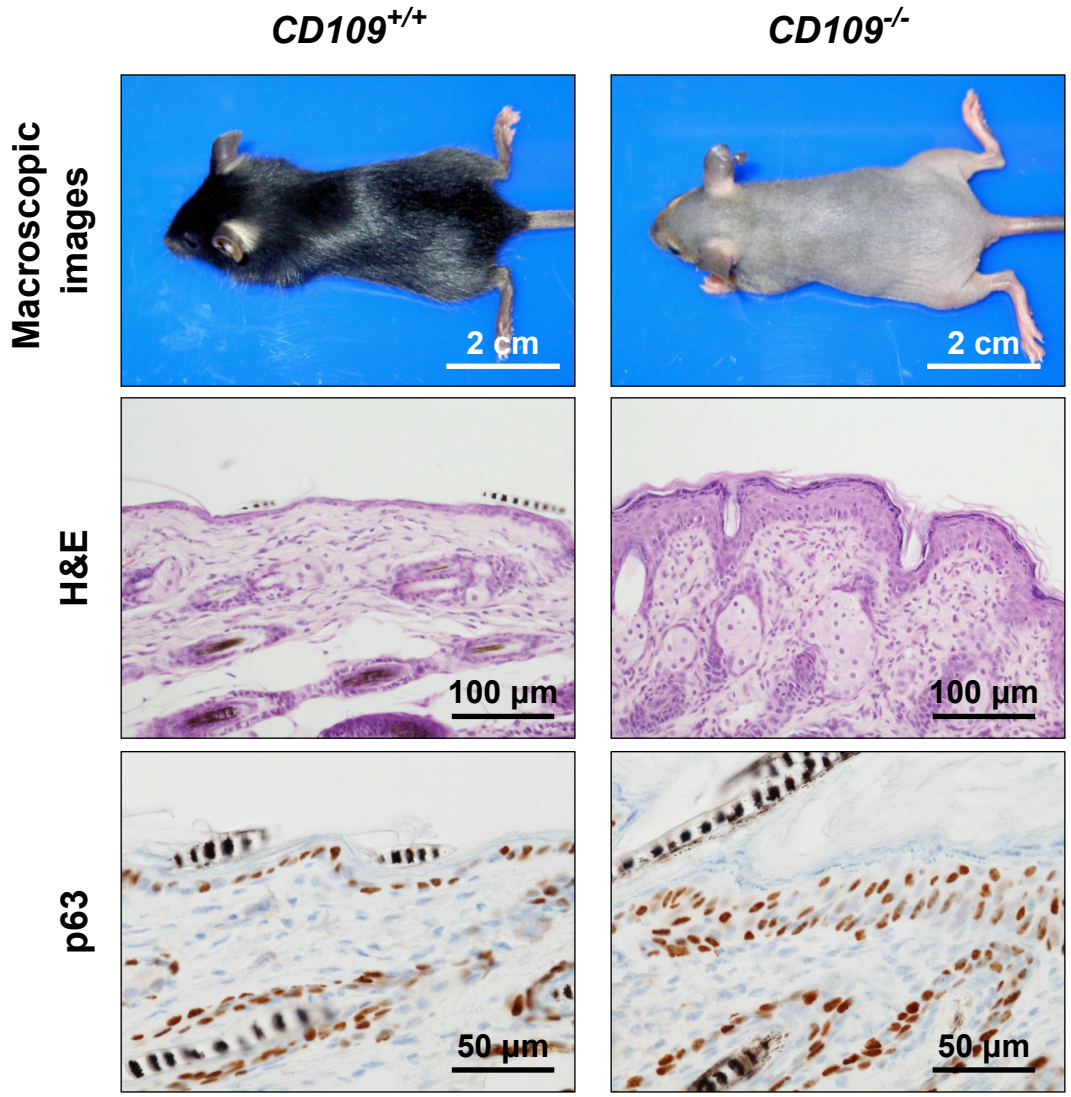
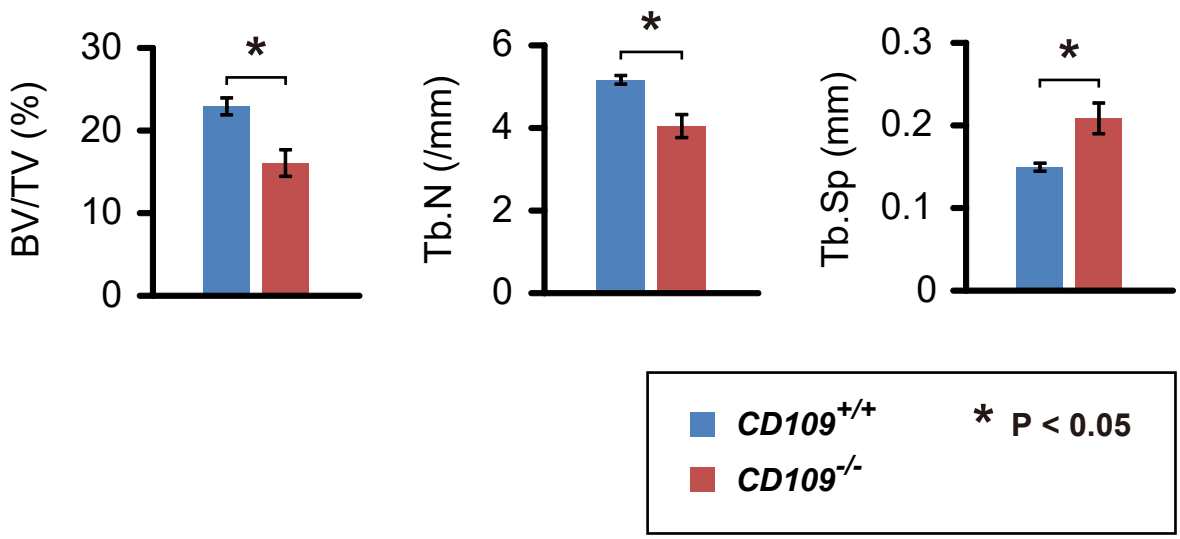
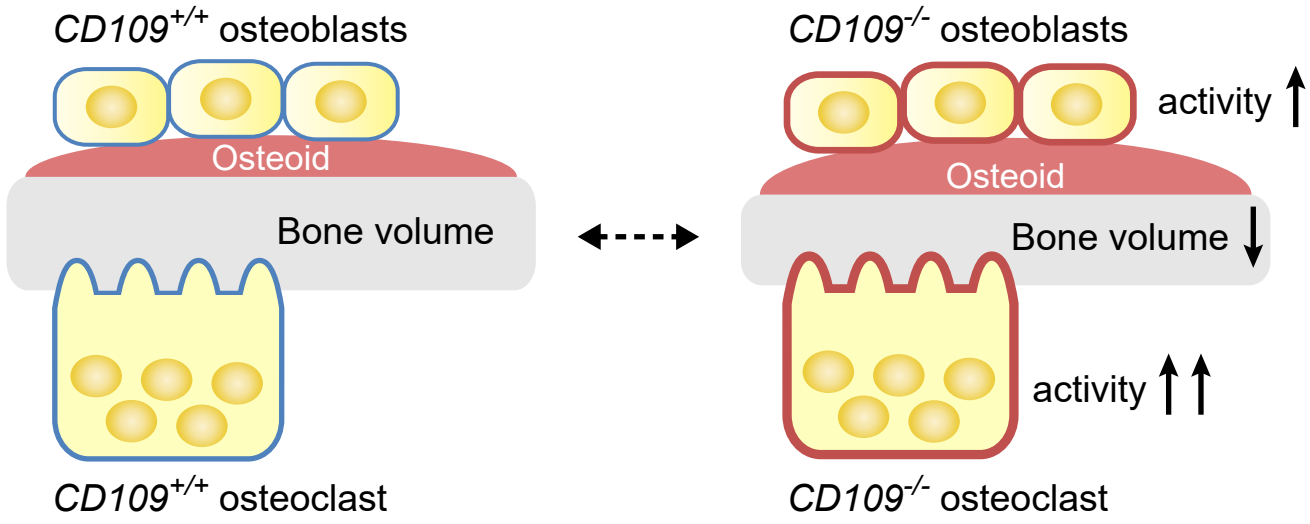

CD109+/+
CD109-/-
Macroscopic images
H&E
p63
2 cm
2 cm
100 µm
100 µm
50 µm
50 µm

Figure 6

a


BV/TV (%)
30
20
10
0
*
Tb.N (/mm)
6
4
2
0
*
Tb.Sp (mm)
0.3
0.2
0.1
0
*
CD109+/+
CD109-/-
* P < 0.05

b


CD109+/+ osteoblasts
Osteoid
Bone volume
CD109+/+ osteoclast
CD109-/- osteoblasts
activity
Osteoid
Bone volume
activity
CD109-/- osteoclast